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Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

General Summary of the Meeting

J. P. Levy

I will not try to summarize the whole meeting, which has covered the whole scope of leukemia research. The progress in therapy has been overviewed by E. Henderson, so that I will limit my summary to the following questions:

1. Have there been any new developments in leukemia etiology?
2. What has been the progress in elucidating the mechanism of malignant transformation of hematopoietic cells?
3. How well do we understand the nature of leukemic cells?
4. What is the role of tumor immunology in leukemia research?

A. Have There Been Any New Developments in Leukemia Etiology?

It now seems clear that multiple factors are involved in the etiology of leukemias and cancers, including X-rays, chemical carcinogens, and viruses, and even that leukemias can also occur by "spontaneous" mutation without the participation of any of these agents. We are no longer looking for *the* human leukemia virus. Curiously enough, this is just the moment when, after repeated misjudgments over 20 years, a C-type virus of probable human origin has been described. What we know about this human T leukemia virus (HTLV) is still limited, but it appears from the presentations of B. Gallo's group that:

1. It must be a C-type retrovirus.
2. It is different from any previously described agent.
3. It is possibly a human virus. Obviously further studies are necessary to establish this point definitely, but the present information supports this conclusion.
4. It human, it is an exogenous virus, not present in the human genome.
5. Several isolates have been characterized in different areas of Asia and America.
6. There are good arguments that it might be a leukemia virus; notably the epidemiology showing a relationship between T lymphomas and the presence of specific antibodies in patients and related people, the sticking association with a pathology of T cells only and, as reported here, the possible in vitro transforming activity of HTLV for human cord blood T cells.

This suggests that two different human malignant hematopoietic disease could be associated with viruses: Pre-B cell malignancies of the Burkitt type with EBV and certain T lymphomas or leukemias with HTLV.

It this is confirmed, several questions will remain to be solved.

First: are these viruses transforming or could they be only promoting factors as possible for EBV. Second: why are these malignancies so infrequent, since they represent only a small percentage of human leukemias? Is it really due, as probable, to the existence of a strong immune response directed against the viral antigens? If yes, the reason why the immune response could be deficient in the clusters of HTLV-associated diseases will remain to be determined as well as the possible role of co-carcinogens. These questions have long been posed with regard to EBV. Another point will be the possibility of vaccinating, which could be difficult for technical as

well as economic reasons. Moreover, how do we decide who should be vaccinated against such an unfrequent disease? Epidemiological studies with the aim of defining high-risk patients and possible co-carcinogens therefore appear very important for the future prevention of these virus-associated malignancies.

B. What Has Been the Progress in Elucidating The Mechanism of the Malignant Transformation of Hematopoietic Cells?

Three groups of information have been presented in this meeting concerning transformation by virus-associated *onc* genes (*v-onc*), by cellular *onc* genes (*c-onc*), and without *onc* gene.

I. Transformation by *v-onc*

That *v-onc* is responsible for the malignant transformation induced by oncogenic viruses is clear, as confirmed in this meeting by the results of Bister et al., for example; but the mechanism of the activity of the 15–20 *v-onc* presently known remains to be established. Some of them produce a protein with tyrosine-phosphorylase activity. Their target protein seems to be related to the cell membrane or cytoskeleton, but we are still ignorant of its precise nature. It has not even been definitely demonstrated that tyrosine phosphorylation is related to carcinogenesis. One may suppose that this kind of *onc* gene product either inhibits the action of a regulator exogenous factor or that it mimics its effects inside the cell. On the other hand, K. Moelling and her colleagues have shown that the *myc* product is a DNA-binding protein, and they reported that the *Erb*^{a} gene product could have a third mechanism of action which involves neither a protein kinase nor a DNA-binding protein. This shows that the malignant transformation might occur as the result of different molecular lesions due to various kinds of *onc* gene products.

It also appears that a common mechanism might exist for DNA- and RNA-virus-induced transformations as suggested by the observation that the *myc* product and SV40T antigen are both DNA-binding proteins, while it has been suggested that a polyoma virus antigen could be a protein kinase like most of the *onc* gene products of RNA viruses. W.S. Rigby has shown that normal cellular proteins are induced by SV40. One may suppose that among these proteins, some are especially important for transformation, and one may imagine that some of them can be involved whatever the inducing virus if a chain of molecular events is altered at different steps by various carcinogens.

II. Transformation by *c-onc*

Several papers have recently suggested that leukemia viruses not possessing an *onc* gene might be leukemogenic by derepressing a *c-onc*. We know that *c-onc* and *v-onc* are very similar and could be identical, as illustrated here by the presentations of F. Wong-Staal et al., Vande Woode et al., and Dalla Favera et al. It has been shown also that *c-onc* can be expressed in experimental as well as human tumors. What does this mean?

In animal species from which *v-onc* and their *c-onc* counterpart have been initially described, the question at first appeared simple, following the observation that the derepression of *c-myc* by an upstream integrated viral LTR able to induce the transformation. As discussed in this meeting, notably by F. Vande Woode and by P. Duesberg, the phenomenon seems less clear now that the viral LTR can be integrated not only upstream, but also downstream to the *c-onc,* reading seems to occur in both directions, and the LTR can be integrated relatively far from the *c-onc.* What is the significance of *c-myc* expression in these condition? Is it really related to cancer? How many genes with possible *onc* characters can be expressed which are not detected because we do not possess their *v-onc* counterpart? The most important question has in fact been discussed by P. Duesberg, i.e., are *c-onc* and *v-onc* truly equivalent? It is generally supposed that they are identical and that quantitative differences in the expression of *onc* genes products are sufficient to explain malignancies. It cannot definitely be excluded, however, that qualitative differences still exist between *v-onc*

and *c-onc*. Minor differences in their sequences, as illustrated by Papas et al., might be responsible for the oncogenic properties of *v-onc*. In addition, the role of the so-called "introns" which exist in *c-onc* and not in *v-onc* might be important for a cellular function of *c-onc* that we are still ignorant of, and it would be very important to know what the normal role of the *c-onc* is in differentiation or for any other function. Are they capable of something which *v-onc* is not? Finally, many *v-onc* produce a protein which is not really equivalent to the *c-onc* product since it is associated with viral sequencies coming from the *gag* gene for example, and we do not know whether this association could modify the function or not. On the whole *c-onc* genes are possibly responsible for cancer due to their quantitatively abnormal expression. Many arguments support this idea, but the possibility still remains that *v-onc* could be the abnormal equivalent of *c-onc,* expressing an oncogenic potency which does not exist for *c-onc*. The observation that *c-mos* associated with a viral LTR becomes oncogenic strongly supports the quantitative hypothesis as shown by Vande Woode, but why *c-src* or *Hv-mos* do not function in the same conditions still needs explaining. It is probable that the problem will not be solved until we know the normal function of *c-onc* genes, which seems to be so conservative that they exist, at least for one of them *c-src* from sponges to human beings, as illustrated by F. Anders. The solution of this problem must be of importance for future developments in cancer therapy.

Another approach of the role of *c-onc* has been reported in this meeting by F. Wong-Staal et al., Della Favera et al., Rübsamen et al., and Vande Woode et al., who have studied the expression of known *c-onc* in human tumors. It seems that *myc, abl,* and *Hv-mos* (the *c-onc* corresponding to the *v-onc* of Harvey virus) can be expressed in any kind of tumor. On the other hand, *myb* was found in poorly differentiated tumors only, *src* was rarely expressed but present for example in some breast cancers, and the expression of *sis* appeared exceptional. It is difficult to make conclusions about the significance of these phenomena, expression being either occasional without clear tissue specificity, or regular in all kinds of tumor. Moreover, normal tissues are able to express the same genes at a relatively high level.

Other groups are looking for *c-onc* genes by transfection of human tumor DNA in NIH 3T3 cells. M.A. Lane and her colleagues have shown that some highly conservative genes might exist in human as well as in murine tumors, with conservation of restriction sites which could be specific for B- or T-cell malignancies, and even more precisely for poorly differentiated, intermediate, or mature cells of each lineage. These genes are different from the known *c-onc* genes which have been tested. On the other hand, Dautry et al. reported the expression of the Harvey gene in bladder carcinoma, that of the Kirsten gene in colonic cancer, and that of another gene in HL 60 leukemic cells and possibly also in Burkitt tumors. HL 60 cells have been shown also to express *c-myc* (Della Favera et al.), which, however, appeared not to be expressed in other acute promyelocytic leukemias. These results are fascinating since they suggest the possible role of at least some of these genes in human malignancies, but their interpretation remains difficult. It has previously been shown by Cooper et al. that the human normal DNA contains genes which are able to transform 3T3 cells. On the contrary, the genes described by M.A. Lane are apparently not found in normal DNA, which could suggest that they are not the exact equivalent of the *c-onc*. On the other hand, such experiments are presently limited by technical problems, and further studies using other target cells from other tissues and other animal species, including man, are necessary for progress. Another question is related to the possible selection in such experiments of *c-onc* genes of which the corresponding *v-onc* have been isolated precisely by their ability to transform murine 3T3 cells. Does their isolation in these conditions really suggest that they play a role in the original human tumor? A larger number of experiments demonstrating tumor specificity of these genes, as suggested by M.A. Lane, would be at least necessary. At the present time, these observations are remarkable, but no conclusion can be drawn. By the way, it can be observed that the observation by Dautry et al. that Harvey and Kir-

sten gene equivalents transform NIH 3T3 cells would support the previously discussed idea that *c-onc* are transforming and qualitatively equivalent to *v-onc*.

III. Transformation Without *onc* Genes

B. Haseltine and P. Fischinger have presented results obtained with murine leukemia virus which suggest possible oncogenic transformation without *onc* genes – more precisely, without a direct intervention of *onc* genes. Weissman has previously suggested that the permanent stimulation of T cells by a C-type virus which is their specific antigen might favor the appearance of leukemia-specific chromosomal abnormalities. Experimental data supporting this idea have been obtained in the group of J. Ihle. The observation by P. Fischinger that there are a very large number of different MCF-tpe *gp* recombinants of the Moloney virus supports the idea that multiple T-cell clones of different specificities might be involved in this phenomenon, perhaps explaining the diversity of leukemia which is produced. On the other hand, the study of AKR leukemia viruses by B. Haseltine and his group shows that the oncogenic potency of one of these agents is related to a very precise mutation near the 3′ end. This suggests something wrong on the intracellular portion of p15E. How can it explain malignancy? Could the proteins of the viral envelope be related to normal cell surface proteins? It has been shown, for example, that p15E of the Moloney virus would be the receptor for $C1_q$, and it is possible that cellular proteins of the gp70 family might be involved in cellular interactions, notably in the thymus. Does an abnormal protein induce abnormal cell interaction with chronic stimulation and eventually the possible induction of *c-onc* or any other genetic abnormality?

In conclusion, it is still impossible to draw conclusions about the mechanism of viral oncogenesis, and even more difficult to propose to general theory of carcinogensis, but the progress has been remarkable in the last 3 years, and such a theory appears at least possible in the next few years.

One must say that in addition to the data obtained by virologists and molecular biologists, very important information has been obtained in the last 3- to 4-year period by cytogeneticists. This point has not been developed in this meeting, but the remarkable advances in chromosome isolations presented by Dr. Young, with the possibility of separating the normal and the translocated chromosomes of one pair, will provide an extremely useful clue in correlating the morphological and biochemical lesions of chromosomes and in determining, in cases where there is a leukemia-specific translocation, which genetic sequences are involved.

Altogether, these advances suggest for the first time that an understanding of what a cancer cell is at the biochemical level will be soon possible.

C. How Well Do We Understand the Nature of Leukemic Cells?

The first point which is now definitely clear is that any leukemic cell has a normal counterpart. This has already been strongly suggested by the recent progress in cytology and pathology, and this is now clearly demonstrated by the use of different markers, including notably monoclonal antibodies as shown by several presentations at this meeting. A remarkable clarification of the classification of the malignant diseases of hematopoietic origin has been recently achieved, as clearly shown here by M. Greaves and also by D. Cooper. Up to recently, however, two cases have remained mysterious: hairy cell leukemia and Hodgkin's disease. As far as hairy cell leukemia is concerned, it appears possible that the normal counterparts of leukemic cells belong to a new minor cellular population of unknown function. Similarly, we have learned here from Dr. Stern and Dr. Diehl that the Reed-Sternberg cell of Hodgkin patients would not belong to any of the previously described lineages. It would be the malignant counterpart of a normal cell present in the external region of lymphoid follicules, as well as in spleen and bone marrow. Since there are now permanent cell lines which are apparently de-

rived from Sternberg cells and specific monoclonal antibodies, it will probably be possible to study the exact nature and function of this new cell, which apparently is not a macrophage but possesses several properties generally supposed to be associated with macrophages, including the production of IL1 and CSF, the expression of Ia antigens, and an accessory cell function in immunological responses. The results reported here are very important for the understanding of Hodgkin's disease, which is the last frequent malignant hemopathy of which the origin remained unclear with so contradictory conclusions from different groups.

It appears not only that leukemic cell lines have a normal counterpart, but also that heir phenotype can be normal, as far as the presently known markers are studied. As pointed by M. Greaves, it is probable that normal progenitors possess all the genetic information necessary for the expression of leukemic properties. The leukemic cells seem remarkable, mainly by an abnormal stabilization of their phenotype at a given stage, with uncoupling of growth and differentiation. The appearence of some phenotypic abnormalities in the leukemic cell is frequent, but it might be a late event. Furthermore, the reversion of leukemic cells to normal cells is possible, and the results reported by Dr. Metcalf suggest a possible reprogrammation of leukemic cells with normal differentiation under the influence of biological soluble factors. This has also been illustrated by M. Moore using the soluble HDIF, and the possible effect of chemical substances like retinoids and dihydroxychole calciferol. From all these observations, it appears that an apparently normal functional adult cell can derive from a leukemic cell. Is this compatible with the results obtained by molecular biologists? The answer is probably yes, since the genetic lesion of malignant cells, whether related to the expression of *c-onc* genes or not, could be finally responsible for an abnormal reaction to soluble factors with uncoupling of growth and differentiation. A continuous treatment by soluble factors would therefore be necessary to maintain the normal differentiation of leukemic cells, which would be cured at the phenotypic but not genotypic level, unless a real reprogrammation of the cells could be induced by soluble factors as suggested here by Dr. Metcalf.

It must be pointed out that we are still almost completely ignorant of the exact reason why a normal cell becomes a leukemic cell. It could be hypersensitive to growth factors, which could also be produced in excess in the surrounding of progenitor cells by the abnormal progenitors themselves or by other cells. One can also imagine that leukemic cells are less sensitive to differentiation factors. The only point which is clear is that this cell is not a monster.

What soluble factors are involved in these phenomena? This is still impossible to answer since we do not know exactly the number and the role of soluble factors in normal granulopoiesis for example. From the presentations of Dr. Metcalf and Dr. Moore, it appears that there is a family of CSF probably acting at several levels, with variable degrees of specificity, but the exact number of these factors is still unclear. Moreover, there is a very important point: are the same or different factors involved in cell growth and cell differentiation? It would be perhaps easier to understand leukemia if different factors were involved, but purification and molecular cloning of the different CSF and related factors will probably be necessary to answer this question. They will also be necessary before hypothetical use of these factors for leukemia treatment. The results presented at this meeting have shown that there is reasonable hope that this hypothesis will be confirmed in the future.

D. What is The Role of Tumor Immunology in Leukemia Research?

At this meeting we have had some excellent presentations in basic immunology. I cannot summarize these papers, which in fact were not directly related to leukemia. One must say, however, that major progress in understanding leukemia and its treatment will probably occur as a consequence of a better knowledge of cell membrane antigens, and the results which have been reported and discussed by H. Ploegh and by

C. Terhorst on the biochemistry of histocompatibility and differentiation antigens, or the progress in the understanding of these antigens at a genetical level, as presented by E. Weiss and by N. Mitchison, are opening up new areas in this research.

The part on specific tumor immunology was not very large at this meeting, and this is not surprising since some disappointments have followed the enthusiastic period that tumor immunology went through some years ago. The research on tumor-specific antigens in human beings has not been very fruitful, and this is in agreement with the observations about the nature of leukemic cells as extensively discussed during these 3 days. It is probably not surprising that no specific antigen exists on tumor cells if these cells have a phenotype similar to that of normal cells, and if they result only from an uncoupling between growth and differentiation. If *c-onc* genes are involved, one can imagine that their products would be nonantigenic for the host. Nevertheless, a virus-specific immune response must exist when a virus is present, and the HTLV-associated leukemias will probably lead to new interest in tumor immunology.

A marginal observation concerning these leukemias has been reported by B. Gallo which deserves further discussion. It seems that they can express foreign class I HLA activity, recalling previously reported observations in murine systems. The remarkable results reported here by E. Weiss on the cloning of *HLA genes* do not support the hypothesis that normally silent histocompatibility genes are depressed in leukemic cells as sometimes suggested. One may imagine minor posttranslational modifications of HLA molecules, or that the association of these molecules with viral products would mimic allospecificities. Whatever its nature, this phenomenon could be useful for leukemia rejection, and it would be interesting to know whether it is specific for virus-associated systems. This was not clear in the murine system due to the high level of contamination by C-type viruses of any murine tumor.

Much attention has been paid in recent years to nonspecific tumor immunology and especially to natural killer cells. Initially known only by their apparently nonspecific activity on tumor cells, they have been progressively better defined morphologically and by their markers in man. Their exact nature however, remains, unclear, and they have recently been described as T-cell precursors, or monocytes, or as a special lineage, and the existence of several kinds of NK cells with different markers has been described. An overview of NK cells has been given here by H. Wigzell, and it appears that besides well-defined NK cells other cells may acquire and NK activity. Cytolytic T cells (CTL), for example, obtained by cloning procedures can be NK cells, but the point is that there are two different structures of these T cells reacting with the target antigen of CTL and the target molecule of the NK activity, respectively. We are still ignorant of this structure that NK cells are able to recognize. From H. Wigzell's data, the situation is less simple than generally supposed: poorly differentiated cells in general are good targets, but the differentiation of these cells can either decrease or increase the sensitivity. Some correlation exists between an increase in the content of sialic acid and glycolipids and a decrease in NK sensitivity. The resistance to NK cells is, however, always relative, and apparently resistant tumor cells can be lysed with stronger NK cells. The main problem remains: we do not know whether NK cells are really protective in vivo against tumors: this is suggested in some cases but not definitely demonstrated. Also we are still ignorant of whether NK cells can have a normal regulatory function, but it appears that they kill CFUs, which can support this fascinating hypothesis.

Finally, a kind of revenge of tumor immunology has been well illustrated during the last day of this meeting. Monoclonal antibodies specific for differentiation antigens expressed normally on leukemic cells represent a new possibility in leukemia therapy, either as vectors of drugs or toxin as shown here for example by P. Thorpe, or to eliminate residual leukemic cells before a bone marrow autograft, as illustrated remarkably by the Sydney Farber Group. On the other hand, bone marrow allografts now represent one of the major components of leukemia treatment, and from the results of Dr. Thomas it is clear that more and more patients will be grafted in

future years. Here again, the progress of basic immunology will become a determining factor since the problem will be to improve the treatment of the graft-versus-host reaction (GVHR), which is the primary cause of death in AML. Nevertheless, the results observed with ALL suggest that GVHR is probably useful in eliminating leukemic cells; we will perhaps have to learn what the benefit of GVH is.

The general conclusion of this meeting is therefore very optimistic. Cancer research has recently seen a relatively black period, but a new period is now beginning. We have at the same time very good progress in the understanding of the leukemic cell at molecular as well as cellular levels, and really new approaches in therapy.

The situation has never been so stimulating for scientists.